Anal. Chem. **1997,** *69,* 2086−2090

Composite Transducers for Amperometric Biosensors. The Glucose Sensor

Jozef Švorc,[†,‡] Stanislav Miertuš,*[,†] Jaroslav Katrlík,[†,‡] and Miroslav Stred'anský[†,‡]

Area di Ricerca, POLY-tech, Padriciano 99, 34012 Trieste, Italy, and Slovak Technical University, Radlinskeho 9, 812 37 Bratislava, Slovakia

A new concept of a composite transducer for amperometric biosensors based on the use of a solid substance with amphiphilic character (called a solid binding matrix, SBM) is presented. The electrochemical properties of the transducers prepared with five different SBMs and the characteristics and performance of SBM-based glucose sensors prepared by three different methods are described. Biosensor stability is evaluated and discussed. The biosensor was used for the determination of glucose in wine, yielding results which were consistent with those obtained with the commercially available Glucose Enzyme Photometric Kit. The average accuracy was 6% for the whole range of analyzed concentrations (0.2−47 g/L) using the same sample dilution in a buffer.

Electrochemical biosensors, especially amperometric ones, have an important position among biosensors.[1−5] Since the late 1980s, intensive research activity has been devoted to the development of amperometric biosensors in conjunction with carbon paste (CP). Exhaustive reviews on CP-based sensors have been published recently,[6,7] where various types of biosensors (e.g., those for glucose, fructose, galactose, ethanol, glycerol, amino acids, lactate, xanthine, etc.) based on related oxidases and dehydrogenases, whole cells, and plant tissues are reviewed. A major advantage of CP-based biosensors is the feasibility of bulk modification of the electrode material with biocatalyst as well as with other components essential for their effective functioning.[6−14] Bulk modification allows sensors to be created with either renewable or disposable surfaces, so that each measurement can be performed on the new surface and not be affected by the residuals from the previous measurement. Other advantages are their low cost, simple preparation, and low background current.

Despite the mentioned advantages of CP-based biosensors, until just recently none has been commercialized, while only one biosensor system based on an artificial mediator (Exactech's glucose pen) is currently marketed.[6] However, industrialization of CP-type biosensors with renewable surfaces by El Murr's group is in progress.[14] The main limitations of CP-based biosensors can be listed as follows: (i) weak mechanical properties due to their creamy texture, which can easily lead to disintegration of the system; (ii) poor reproducibility of the electrode fabrication; (iii) leakage of mediator out of the CP; and (iv) failure of electron transfer from the biocatalytic site to graphite.

The properties of CP-based biosensor can be partially improved by the incorporation of additives into the paste, e.g., polyethylenimine, acetylenic polymers, chitosanglutamate, cationic antibiotics,[15] trehalose,[16] and fumed silica.[17] To prevent the leakage of mediator, covalent binding of the mediator to a polymer matrix[13,18,19] or direct modification of enzymes with mediator has been used.[20] The approaches to improve the properties of CP mentioned above have certain limitations and deficiencies. On one hand, it is still unclear how additives might affect the biosensor performance at the molecular level, thus making the search for suitable additives for each specific biosensor rather difficult and more random than systematic. On the other hand, covalently bound mediators exhibit modified electrochemical properties and reduced mobility, which affect the reaction rate with enzymes.

Recently, we presented a new concept of composite transducers for amperometric biosensors with improved molecular environment within the transducer body, based on the use of solid components instead of pasting oil.[21] Such compounds (called solid binding matrices, SBMs) are solid at room temperature, have a suitable melting point, and have amphiphilic character, although they are prevalently hydrophobic. They show a beneficial effect on the mechanical and electrochemical properties of the final

[†] POLY-tech.

[‡] Slovak Technical University.

(1) *Biosensors. Fundamentals and Applications*; Turner, A. P. F., Karube, I., Wilson, G. S., Eds.; Oxford University Press: Oxford, UK, 1987.
(2) *Biosensors. A Practical Approach*; Cass, A. E. G., Ed.; IRL Press: Oxford, UK, 1990.
(3) Buerk, D. G. *Biosensors. Theory and Applications*; Technomic Publishing: Lancaster, PA, 1993.
(4) *Proceedings of the Conference on Trends in Electrochemical Biosensors*; Costa, G., Miertus, S., Eds.; World Scientific Publishing: Singapore, 1992.
(5) *Food Biosensors Analysis*; Wagner, G., Guilbault, G. G., Eds.; Marcel Dekker: New York, 1994.
(6) Gorton, L. *Electroanalysis* **1995**, *7*, 23−45.
(7) Kalcher, K.; Kauffmann, J.-M.; Wang, J.; Svancara, I.; Vytras, K.; Neuhold, C.; Yang, Z. *Electroanalysis* **1995**, *7*, 5−22.
(8) Kulys, J.; Wang, L.; Hansen, H. E.; Buch-Rasmussen, T.; Wang, J.; Ozsoz, M. *Electroanalysis* **1995**, *7*, 92−94.
(9) Smolander, M.; Marko-Varga, G.; Gorton, L. *Anal. Chim. Acta* **1995**, *302*, 233−240.
(10) El Atrash, S. S.; O'Neill, R. D. *Electrochim. Acta* **1995**, *40*, 2791−2797.
(11) Chi, Q.; Dong, S. *Anal. Chim. Acta* **1994**, *285*, 125−133.
(12) Boujtita, M.; Chapleau, M.; El Murr, N. *Anal. Chim. Acta* **1996**, *319*, 91−96.
(13) Pravda, M.; Adeyoju, O.; Iwuoha, E. I.; Vos, J. G.; Smyth, M. R.; Vytras, K. *Electroanalysis* **1995**, *7*, 619−625.
(14) Boitard, M.; Chapleau, M.; El Murr, N. *Proceedings from the Fourth World Congress on Biosensors*; Elsevier: Oxford, UK, 1996; p 35.
(15) Lutz, M.; Burestedt, E.; Emnéus, J.; Lidèn, H.; Gobhadi, S.; Gorton, L.; Marko-Varga, G. *Anal. Chim. Acta* **1995**, *305*, 8−17.
(16) Kulys, J.; Hansen, H. E. *Anal. Chim. Acta* **1995**, *303*, 285−294.
(17) Wang, J.; Liu, J. *Anal. Chim. Acta* **1993**, *284*, 385−391.
(18) Gorton, L.; Karan, H. I.; Hale, P. D.; Inagaki, T.; Okamoto, Y.; Skotheim, T. A. *Anal. Chim. Acta* **1990**, *228*, 23−30.
(19) Hale, P. D.; Lan, H. L.; Boguslavsky, L. I.; Karan, H. I.; Okamoto, Y.; Skotheim, T. A. *Anal. Chim. Acta* **1991**, *251*, 121−128.
(20) Karube, I.; Clark, S. A.; Nagata, R. European Patent EP 0 563 795 A1, 1993.
(21) Švorc, J.; Miertuš, S.; Stred'anský, M. PCT Int. Patent Application MI95A001441, 1995.

S0003-2700(96)00948-1 CCC: $14.00

transducer, secure molecular communication between the redox centers of biocatalysts and mediators. Similar approaches were also used recently by other authors.[22−24]

In this paper, the electrochemical properties of the transducers prepared with five different SBMs and the analytical characteristics and performance of the glucose sensor based on these transducers are presented. Three different procedures for biosensor preparation are also described and evaluated.

EXPERIMENTAL SECTION

Materials. Glucose oxidase (GOx, type VII-S), cholesteryl oleate (CO), and cholesteryl myristate (CM) were obtained from Sigma (St. Louis, MO). Hexadecanol (HXOL) and hexadecanone (HXNE) were from Fluka (Buchs, Switzerland). Ferrocene (FC), 1,1′-dimethylferrocene (DMFC), tetrathiafulvalene (TTF), and synthetic graphite powder (Catalog No. 28,286-3) were purchased from Aldrich (Steinheim, Germany). 1-Monostearoyl glycerol (MG) was a gift from Dr. Marcincin, Slovak Technical University, Bratislava, Slovakia. Other analytical grade reagents were commercially available.

Apparatus. Cyclic voltammetric studies were carried out with the computerized electrochemical analyzer AMEL 433/W (Milan, Italy). Chronoamperometric studies were carried out with a potentiostat (AMEL 559) and a recorder (AMEL 868). A saturated calomel electrode (SCE) and a Pt spherical electrode were always used as the reference and the counter electrodes respectively.

Preparation of Composite Transducers. The graphite powder was first modified with a mediator (FC, DMFC, or TTF). Next, 0.5 g of mediator-modified graphite was usually prepared. The desired amount of mediator (6 or 7% w/w) was dissolved in chloroform (5−6 mL). Then, the graphite was added and the mixture stirred vigorously with a magnetic stirrer until about half the chloroform volume was evaporated. The suspension was then transferred and spread on the bottom of a glass Petri dish (d = 10 cm). The solvent was allowed to evaporate in a oven at 40−45 °C.

The composite transducer was prepared by three different methods:

Preparation Method A. The mediator-modified graphite was thoroughly mixed with a SBM in a mortar. Then, 50% (w/w) of the SBM with respect to the final weight of the composite material was used. A part of the obtained composite material (15−20 mg) was packed in a PVC tip (i.d. 2.0 mm, o.d. 5 mm, length 20 mm) and manually pressed. A cyclinder (height 3−4 mm) was formed inside the tip. Electrical contact was ensured by use of a brass rod. The active part of the transducer was polished on a sheet of paper.

Preparation Method B. The preparation was done as described for method A, except that the last mixing was carried out with addition of chloroform (100 μL/100 mg of the material) to ensure homogeneous mixing.

Preparation Method C. The SBM (200 mg) was melted in a porcelain dish immersed in an oil bath heated at 5−10 °C above its melting point. The mediator-modified graphite (150 mg) was then added and mixed well. The same PVC tip as described above was used as a holder of the electrode. The brass rod was inserted into the tip to create a cylindrical space (height about 2−3 mm). The space was filled with the melted mass. The electrode was left to cool at room temperature. After cooling, the excess material was cut out on sandpaper. The surface was then smoothed on a sheet of a paper.

Preparation of Glucose Biosensor Using a Layer of GOx. One microliter of a water solution of glucose oxidase (20 or 100 mg/mL) was applied on the surface of the composite transducer and spread onto the whole surface. After drying at room temperature, the surface was covered with the dialysis membrane (Spectra/Por, type 1 (Spectrum Medical Industries Inc., Houston, TX), secured with an O-ring.

Preparation of Bulk Modified Glucose Biosensor. The graphite containing the mediator was first modified with GOx. The enzyme (5% w/w with respect to the final weight of the composite material) was dissolved in water, and then the graphite was added, the suspension thoroughly mixed, and water evaporated under reduced pressure. The obtained mass was then mixed with the SBM and packed in the same way as described in Preparation of Composite Transducers. The active surface of the biosensor was covered with the dialysis membrane using an O-ring.

Measurements by Biosensors. All experiments were carried out at 25 °C in a jacketed reaction vessel, equipped with magnetic stirring. The biosensor and the reference and counter electrodes were immersed in a 0.2 M phosphate buffer (5 mL). Some measurements were done in the absence of oxygen, which was removed by bubbling nitrogen. After current stabilization, a standard glucose solution or sample was added, and current−time response curves were recorded. The height of the recorded wave (current increase) was correlated to the concentration of glucose.

RESULTS AND DISCUSSION

The aim of the present study was to improve both the mechanical properties and the performance of the transducers and, consequently, the properties of biosensors based on them. A strategy to achieve the mentioned improvements is based on the hypothesis that better mechanical properties could be guaranteed by the use of a solid substance instead of pasting liquid for the preparation of CP-based transducers and biosensors. The appropriate selection of a SBM could also ensure better storage stability of the biosensors. It is known that a suitable environment can significantly improve the stability of enzymes,[25] e.g., many of them are stable in nonaqueous media,[26] and most of them are more stable in the solid state.[25]

Criteria for the selection of a suitable SBM were (a) a suitable melting point of the SBM to ensure the solid compact state of transducers during the storage period and measurement, and at the same time to prevent thermal inactivation of the biocatalyst during biosensor preparation when it is mixed with the SBM in the melted state; (b) a hydrophobic skeleton of its molecule to guarantee insolubility in water; and (c) amorphous character of the SBM to reach a sufficient homogeneity of the transducer components. Among the many existing groups of SBMs[21−24] fulfilling the above-mentioned requirements, we concentrated our attention on those with amphiphilic character, which are compat-

(22) Petit, C.; Kauffmann, J.-M. *Anal. Proc.* **1995**, *32*, 11−12.
(23) Petit, C.; Gonzales Cortes, A.; Kauffmann, J.-M. *Talanta* **1995**, *42*, 1783−1789.
(24) Spohn, U.; Narasaiah, D.; Gorton, L. *Electroanalysis* **1996**, *8*, 507−514.
(25) Li, S.; Schöneich, C.; Borchardt, R. T. *Biotechnol. Bioeng.* **1995**, *48*, 490−500.
(26) Saini, S.; Hall, G. F.; Downs, M. E. A.; Turner, A. P. F. *Anal. Chim. Acta* **1991**, *249*, 1−15.

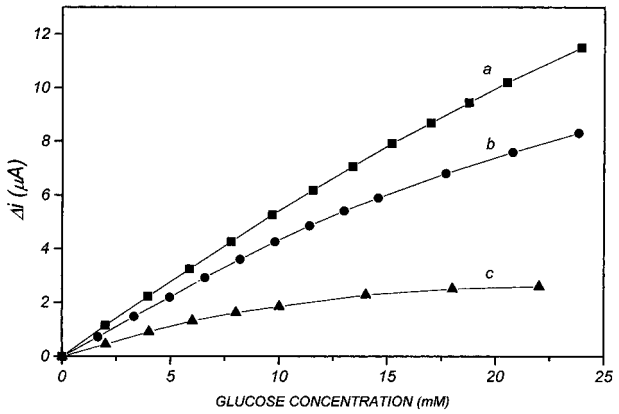

Figure 1. Dependencies of the current changes on glucose concentration of the bulk glucose biosensor based on mixing of solid composites. MG was used as the SBM, and TTF (a) DMFC (b), and FC (c) were added as the mediators. Conditions: oxygen-free 0.2 M phosphate buffer, pH 7; working potential of 300 mV vs SCE.

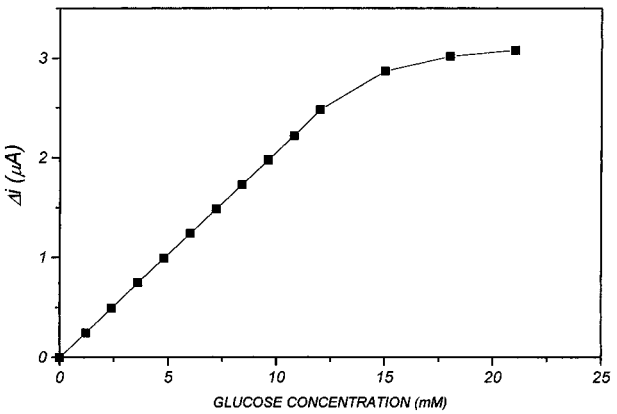

Figure 2. Dependence of the current change on glucose concentration of the sensor based on melted CM, FC, and the layer of GOx. Conditions: 0.2 M phosphate buffer, pH 8; working potential of 300 mV vs SCE.

ible with both hydrophilic and hydrophobic parts of biocatalysts and mediators, thus contributing to a better performance of biosensors. Here we present the results obtained with five types of SBMs: hexadecanol (HXOL, mp 43−46 °C), hexadecanone (HXNE, mp 43−45 °C), monostearoyl glycerol (MG, mp 78−81 °C), cholesteryl myristate (CM, 77−80 °C), and cholesteryl oleate (CO, mp 48−50 °C).

Electrochemical Characteristics of the Transducers. The electrochemical properties of the composite transducers were evaluated by cyclic voltammetry. The voltammetry was carried out in 0.2 M phosphate buffer, pH 7.0, at room temperature without removing oxygen in the potential range from −200 to +600 mV at a scan rate of 100 mV/s. The anodic peak potentials for the transducers containing DMFC at the final contents of 3% and 50% w/w of MG, CM, or CO, prepared by mixing the solid components either in the absence or in the presence of chloroform, were observed at 220−240 mV vs SCE, and the peak separation was 100−120 mV. The anodic peak potentials were shifted by about 100 mV toward positive values in comparison to values obtained using a glassy carbon electrode. A similar potential shift was also found for FC and TTF. The situation changed when modified graphite was mixed with the melted SBM (see preparation method C in the part on Preparation of Composite Transducers). In this case, the anodic peak potentials for DMFC-based transducers were observed at 140−150 mV vs SCE, and the peak separation was 80−90 mV for all five SBMs used. This improvement is due to more intimate contact of components and better homogeneity within the transducer body.

Characteristics of Glucose Biosensors Based on the Composite Transducers. Figure 1 shows dependencies of the current change on glucose concentration for bulk glucose sensors prepared by mixing solid components in the absence of chloroform (see preparation method A in the part on Preparation of Composite Transducers) using MG as the SBM and DMFC, FC, and TTF as mediators. In all cases, the final content of GOx was 5%, the mediator 3%, and the SBM 50% (w/w). The working potential was set at 300 mV vs SCE, and measurements were carried out in pH 7 phosphate buffer in the absence of oxygen. The sensitivity of the biosensor based on TTF was the highest and reached the value of 17.1 μA mM^{-1} cm^{-2}. This can be attributed to the most suitable working potential of the TTF-based transducer (190 mV vs SCE). The sensitivity of the FC-based biosensor was the lowest (6.8 μA mM^{-1} cm^{-2}). This was probably caused by the low working potential with respect to the anodic peak potential of the FC-based transducer (320 mV vs SCE). Moreover, the rate constant of the reaction of ferricinium ions with GOx is higher for DMFC than for FC.[1] The sensitivity of the biosensor that uses DMFC as the mediator was 14.2 μA mM^{-1} cm^{-2}. For other SBMs, the sensitivity of the glucose biosensors was approximately 3 times lower in comparison with MG-based biosensors. The upper linearity limits of the prepared biosensors were 5 mM for FC, 10 mM for DMFC, and 12 mM for TTF. These linearity limits are in the range of the upper linearity limits (1−100 mM) of the CP/GOx-based glucose biosensors.[6] The response time of all biosensors was about 1−2 min.

Figure 2 shows the dependence of the current change on glucose concentration for the biosensor based on the transducer prepared by mixing FC-modified graphite with melted CM and the layer of GOx. The content of the mediator in the transducer was 3%, and the total activity of GOx in the layer was 3.5 units. The working potential was set at 300 mV vs SCE, and measurements were carried out in pH 8 phosphate buffer without removing oxygen. Under these conditions, the sensitivity achieved the value of 6.5 μA mM^{-1} cm^{-2}. The upper linearity limit was 12 mM. The response time of the biosensor was the same as for the bulk ones. For the presented type of biosensors, we found a decrease in sensitivity with increasing thickness of the GOx layer. When glucose passes through the thicker biocatalytic layer, it interacts with GOx, and the enzyme is preferably regenerated by the consequent reaction with oxygen instead of the mediator because the mediator is too far from the site of the biocatalytic process, which results in the decrease in the sensor sensitivity.

Figure 3 shows dependencies of the current change on glucose concentration for biosensors prepared by admixing of FC−GOx-modified graphite into the melted CM, HXOL, and HXNE, respectively. The working potential was set at 300 mV vs SCE, and measurements were carried out in pH 8 phosphate buffer without removing oxygen. The sensitivities of all biosensors are similar (5.8 μA mM^{-1} cm^{-2} for CM-, 4.9 μA mM^{-1} cm^{-2} for HXNE-, and 4.1 μA mM^{-1} cm^{-2} for HXOL-based biosensors), and they are comparable with the sensitivity of the biosensor based on the melted CM and GOx layer. The upper linearity limits are 5−6 mM. It is interesting that this type of biosensor is functioning in spite of the thermal stress on the enzyme during the preparation,

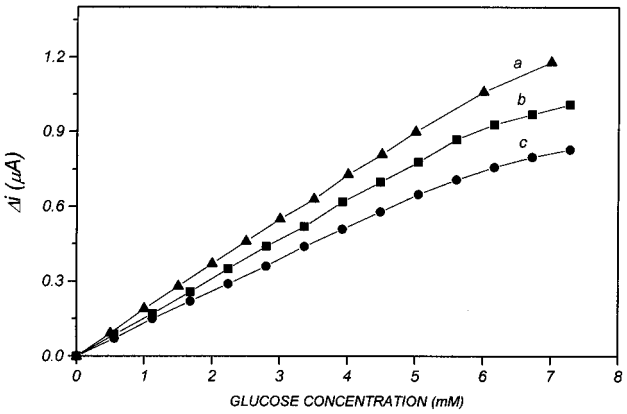

Figure 3. Dependencies of the current changes on glucose concentration of the sensor prepared by admixing of FC−GOx-modified graphite into melted CM (a), HXNE (b), and HXOL (c). Conditions as in Figure 2.

especially when CM is used (the enzyme was mixed with the melted SBM, heated for few minutes to 90 °C for CM, as compared to 50 °C for HXOL and HXNE). This is caused by the stabilizing effect of the SBM, which is analogous to the effect of other nonaqueous media on enzymes' stabilities. Although GOx is known as an inherently stable enzyme, we have observed the same effect also for less stable enzymes, like alcohol dehydrogenase and diaphorase.[21]

Reproducibility in Fabrication of Biosensors. One of the main problems of CP-based biosensors is the insufficient reproducibility in their fabrication,[6] because it is difficult to obtain homogeneous distribution of all components. Here we present and compare the reproducibilities of biosensors prepared using three fabrication procedures, namely mixing all solid components either in the absence or in the presence of the solvent (chloroform) and admixing the components into the melted modifiers. For each procedure, the same batch of the composite mixture was used. The evaluation of the reproducibility of the biosensors fabrication was based on statistical evaluation of the sensitivities values. The standard relative deviation (s_r) of the values of sensitivities of the biosensors prepared by mixing solid components in the absence of chloroform was the highest and reached 16−21% ($n = 5$). No significant improvement was found for the sensors prepared by mixing the solid components in the presence of chloroform. Better reproducibility was obtained for the biosensors based on melted SBM, the s_r being in the range 11−16%. The best reproducibility was found for the biosensor prepared by mixing FC-modified graphite with melted CM and the layer of GOx ($s_r = 8\%$). Improved reproducibility can be expected from using a better technological process for the electrode preparation with a thorough homogenization. On the other hand, the use of a glucose standard for measuring real samples eliminates the problems of an insufficient reproducibility in biosensor fabrication.

Operational and Storage Stability of the Biosensors. The continuous of operational stability of the bulk glucose biosensor based on MG and DMFC prepared by mixing the solid components (preparation method A) was determined by continuous polarization of the biosensor by 300 mV vs SCE in the presence of 4 mM glucose. During the first 2 days, no significant decrease of the sensitivity was observed. From the third day, the sensitivity gradually decreased, and after 5 days, 40% of the original sensitivity was found. The storage stability of the presented biosensor was at least 3 months at room temperature.

Table 1. Results of the Determination of Glucose in Wine

type of wine	μ (g/L)[a]	c (g/L)[b]	c_1 (g/L)[c]
white (I)	0.2	0.21	0.63
		0.23	0.56
red (II)	0.83	0.62	1.33
		0.73	1.27
white (III)	1.61	1.62	1.93
		1.60	1.82
white (IV)	46.61	46.3	46.3
		45.7	45.7
red (V)	35.17	34.4	34.4
		33.7	33.7

[a] μ, content of glucose determined by use of the Glucose Enzyme Photometric Kit. [b] c, content of glucose determined by use of the glucose biosensor as the result of the postdifferential measurements. [c] c_1, content of glucose corresponding to the total response of biosensor (including interference).

When the transducer based on CM and FC was prepared by method C and the layer of GOx was applied on its surface, the biosensor was functional for at least 20 reproducible measurements. After 6 months of storage at 4 °C, 82 ± 10% of the original sensitivity was detected for this type of biosensor.

The stability of the bulk-modified biosensor prepared by admixing FC−GOx-modified graphite into the melted CM was 1 day. However, those prepared with HXOL and HXNE showed a much higher stability: 40% of the original sensitivity was found after 1 month. The initial sensitivity of all three types of biosensors was detected after as long as 5 months, if the surface of the bulk sensor was reactivated by grinding and smoothing. Thus, it can be concluded that the excellent stability of the enzyme inside the biosensor is due to the protective effect of the SBM against environmental deactivating factors, like oxygen and humidity. The enzyme in the surface layer is much more rapidly deactivated than that inside the composite, especially when a SBM with a high melting point is used (e.g., for CM). This is probably due to a combined effect of heat during the preparation and the consequent effect of the outer environment (humidity, oxygen). From this point of view, the use of SBMs with lower melting points 30−50 °C (e.g., HXOL, HXNE) for bulk biosensors is preferred.

Application of the Biosensors for the Determination of Glucose in Wine. The glucose biosensor based on the transducer with CM and FC, prepared according to procedure C, and the layer of GOx was used for the determination of glucose content in wine. The reliability of the sensor was first tested in a standard solution with 10.0 g/L glucose. For 10 measurements, the mean determined concentration was 10.14 g/L, with a standard relative deviation of 1.8%, and the percentage accuracy was 1.4%. Then, glucose was determined in five samples of wine. The data, compared with those obtained with the Glucose Enzyme Photometric Kit (Boehringer, Mannheim, Germany), are given in Table 1. For low glucose concentrations, especially in the red wines, we have found a significant difference between the contents of glucose determined by the biosensor and those measured with the kit. This difference is caused by the interference of electroactive compounds present in wine. Easily oxidable phenolic acids, antocyanins, catechins, flavonoids, and polyhydroxyphenols occur in red wines at a substantially higher concentration than in white

wines.[27] The presence of interfering compounds was confirmed using the transducer instead of the complete glucose sensor. Significant current increase was found upon red wines additions, and it was dependent on the wine type. It was also found that use of the membrane dramatically reduced the signal caused by interference. For example, for some kinds of red wine, the use of the membrane decreased the interfering signal more than 100-fold, while the sensitivity to glucose decreased only by a factor of 2.5 (the factor value was found for a bulk-modified sensor). Although the membrane significantly reduced interference, high inaccuracy of results for low glucose contents in wine was found. Therefore, postdifferential measurements were used, i.e., the content of glucose determined by the glucose sensor was corrected by the response of the sensor where GOx was replaced by albumin. In this case, a good agreement of the glucose contents with the photometric data was obtained. It should be stressed that the biosensor was used for the determination of glucose in samples with a large range of glucose concentrations (0.2−47 g/L), using the same dilution of the samples in the buffer (1:50). In spite of that, the average percentage accuracy is 6% for the whole range. The results of two independent measurements are summarized in Table 1.

(27) Drdak, M.; Altamirano, R. C.; Rajniakova, A.; Simko, P.; Malik, F.; Balik, J.; Benkovska, D. *Mitt. Klosterneuburg* **1991**, *41*, 190−193.

CONCLUSIONS

A new concept of composite transducers for amperometric biosensors based on solid binding matrices is presented, together with the properties and performance of glucose biosensors based on them. The SBM exhibits better mechanical properties, especially compactness and plasticity, in comparison with the traditional CP-based biosensor. It also provides a suitable molecular environment within the transducer body, which affords efficient communication between the redox center of the biocatalyst and the mediators, effectiveness of the biocatalytic process, conductivity, and especially the stability. The presented concept allows easier industrialization, as supported by the recent development of a portable system for the evaluation of wine quality, in collaboration with Biofutura s.r.l., Italy. At present, glucose, fructose, ethanol, lactate, and malate biosensors are being introduced into the market.

Received for review September 17, 1996. Accepted January 29, 1997.[⊗]

AC9609485

[⊗] Abstract published in *Advance ACS Abstracts*, April 1, 1997.